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RESEARCH ARTICLE

BUCCAL CYTOME ASSAY – A NON INVASIVE SCREENING METHOD FOR EVALUATION OF RADIATION EXPOSURE IN COMPUTER AND MOBILE PHONE USERS

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ABSTRACT

The buccal micronucleus cytome assay is a simple non invasive method used to assay DNA damage. It is well recognized that electromagnetic fields can affect the biological functions of living organisms at both cellular and molecular level. The potential damaging effects of electromagnetic fields and very low frequency and extremely low frequency radiation emitted by computer cathode ray tube (CRT) video display monitors (VDMs) has become a concern within the scientific community. We studied the effects of occupational exposure to VDMs in 119 occupationally exposed to VDMs and 101 unexposed control subjects matched for age and sex with no history of dental ailments were recruited. Genetic damage was assessed by examining the frequency of micronuclei in exfoliated buccal cells Buccal smears obtained from the subjects were analyzed for nuclear anomalies using the buccal cytome assay. Higher degree of karyolytic cells was observed in exposed male (KL) (13.02±0.14), and females (9.81±1.04). Smokers possessed higher nuclear anomalies than the non smokers.

INTRODUCTION

Computers are widely used in recent years in almost all offices, colleges, universities and homes today, approximately 90 million adults use computers regularly worldwide. Computers (Sen and Richard son 2002) emit ionizing and non- ionizing radiation. These include infrared, ultra violet, visible light, X ray and radio frequency emissions (Ivancsits *et al.*,, 2002). The possible health hazards of chronic exposure to extremely low frequency (ELF) and very low frequency (VLF) electromagnetic

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radiation from various appliances including computer cathode ray tube (CRT) video display monitors (VDMs). Over the past few decades several studies have found higher prevalence of computer vision syndrome, glaucoma, Eye strain, musculoskeletal disorder (Chiemeke 2007 *et al.,* Tatemichi *et al.,* 2004, Nakaishi 1999 *et al.,* Cook 2000 *et al.,* Flodgren *et al.,* 2007, Thorn *et al.,* 2007, Blatter *et al.,* 2002 and Grace Pui Yuk Szeto *et al.,.,* 2009). Many workers obtain a large amount of information from visual display terminals (VST's) during general duties as well as whole processing specific jobs.

The quantification of micronuclei by the micronucleus test (MT) is an important non-invasive cytogenetic method which is a good indicator of chromosome mutation (Majer *et al.,* 2001) and has been extensively used for monitoring populations exposed to known mutagens and carcinogens. The major advantages of the micronucleus test over other techniques are that it can be applied to interphase cells and does not require cell culture or the preparation metaphase cells, a further advantage being that because of the low cost of this test it is suitable for the large-scale screening of populations (Holland *et al.,* 1994).

Epidemiological studies have suggested that long term exposure to radiation increases the risk of certain types of cancer, including leukemia, central nervous system cancer, and lymphoma (Wertheimer *et al.,* 1979; Li C Y *et al.,* 1997), skin problems, spontaneous abortions and ocular disorders (Blehm *et al.,* 2005) depression, miscarriages, headaches, insomnia, anxiety (Yan *et al.,* 2008). Over the years the entire world has witnessed an increased usage of mobile phones, radar installations and microwave Ovens resulting in alarming rates of human exposure to radio frequency waves. Mobile phones use microwaves as carrier waves in a frequency range between 300 megahertz to 300 gigahertz. Microwaves possess genotoxic effect to somatic cells of human system and also lead to inheritable genotoxic effects in germ cells (Verschaeve, 2005).

MATERIALS AND METHODS

Subjects

The study was carried out on 119 computer users who were occupationally exposed to CRT VDMs and 101control subjects matched for age and sex. At least one year of working was the criteria used for sample selection of the exposed individuals. The control group with no exposure to any CRT VDMs such as Yoga trainers, Fitness coaches, people from rural areas and tailors for whom usage of computers was very occasional or not necessary. Participants were informed about the objectives of the study. They were asked to sign an informed consent form and to complete a questionnaire to obtain necessary information on their lifestyle and personal factors (age, working period, smoking habits and health, etc).

Buccal cell sampling, preparation and staining

Buccal cells originate from a multilayered epithelium that lines the oral cavity. Prior to buccal cell collection the Computer users and control groups were advised to rinse their mouth thoroughly with water to remove unwanted debris. Sterile wooden spatula was used to obtain cell samples from buccal mucosa. The buccal mucosa was transferred into eppendorf tubes with Phosphate Buffered Saline (PBS) at pH. 7.0 and centrifuged for 10 min at 1500 rpm. Supernatant was removed and replaced with 5 ml of fresh PBS solution and centrifuged for 10 min at 1500 rpm.

This process was repeated thrice, because the PBS helps to inactivate endogenous, DNAases and aid in removing bacteria that may complicate scoring. After discarding the supernatant the pellet was smeared on to clean microscopic slides. Smears were air dried for 10 min, and then fixed in cold methanol: acetic acid (3:1) for 10 min. Slides were air dried for 10 to 15 min and stained in 2% Giemsa for 10 min and rinsed with double distilled water, air dried and viewed under a light microscope (Stitch and Rosin, 1984).

Scoring criteria for buccal cytome assay

Three slides were prepared from each sample. Nuclear abnormalities were classified according to Tolbert *et al.,* (1992).These criteria are intended to classify buccal cells into categories that distinguish between "normal" and "abnormal" based on their aberrant nuclear morphology. These abnormal nuclear morphologies are due to DNA damage and induced cell death. Photographic images showing distinct cell populations were scored in the buccal cytome assay are presented below in Figure 1.

Scoring method

1000 cells were scored per subject to determine the frequency of the various cell types outlined in the buccal cytome assay. This consisted of micronuclei cell, binucleated cells, karyorrhectic cells, and karyolytic cells. A total of 1000 differentiated cells were scored in order to determine the frequency of micronuclei. Cells were scored by using both bright and low field.

Statistical analysis

The slides were coded during processing and decoded at the time of Statistical analysis. The data an each parameter for each group were pooled and Mean ± SD was calculated. The results were statistically analyzed by student't' test along with the Pearson correlation and Spearman's correlation (non-parametric correlation) with the help of statistical software SPSS 13.0.

RESULTS

Table 1 explains the male and female (both exposed and control) subjects below 25 years of age and above 25 years of age. The main characteristics of the exposed and control group (duration of exposure, smoking habits, cell phone usage and habit of wearing glass, Computer Vision Syndrome) are summarized in Table 1. Of the 119 computer users, 92 subjects were both computer and cell phone users and 27 subjects were computer users but not cell phone users. A total of 220 subjects including controls in the age group 19–38years have been studied. Out of these 112 subjects were males (50.90%) and 108 subjects (49.09%) were females. The mean duration of exposure period was 6.96 years (range 4 years to 13 years) and the mean duration of daily exposure was 5.39 hours (range 3hrs to 10 hrs). Among these

Table 1. General Characteristics of study group and control matched for age and sex

S. No	Variables	Exposed (n=119)	Control (n=101)
1	male subjects		
	Group 1 (<25yrs)	27 (22.68)	20 (19.80)
	Group 2 (>25 yrs)	36 (22.68)	29 (28.71)
2	female subjects		
	Group 1 (<25yrs)	21 (17.64)	24 (23.76)
	Group 2 (>25 yrs)	35 (29.41)	28 (27.72)
3	Sex		
	Females	56 (47.05)	52 (51.48)
	Males	63 (52.94)	49 (48.51)
	Smoking Habit		
4	Non smokers	88 (73.94)	71 (70.29)
	Smokers	31 (26.05)	30 (29.70)
5	Cell phone users		
	Females	40(33.61)	28(27.72)
	Males	52(43.69)	41(40.59)
6	Cell phone non users		
	Females	16(13.44)	21(20.79)
	males	11(9.24)	11(10.89)
7	Radiation exposure to computer monitors		
	(CRT/VDM) in male	4.82	NA
	subjects	7.81	NA
	Avg. duration of exposure	6.18	NA
	(years) <6 year	8.24	NA
	Avg. duration of exposure (years) >6 year		
	Avg. duration of daily exposure (hours) <6 year		
	Avg. duration of daily exposure (hours) > 6 year		
8	Radiation exposure to computer monitors		
	(CRT/VDM) in female	4.38	NA
	subjects	7.48	NA
	Avg. duration of exposure	6.30	NA
	(years) <6 year	7.52	NA
	Avg. duration of exposure (years) > 6 year		
	Avg. duration of daily exposure (hours) <6 year		
	Avg. duration of daily exposure (hours) > 6 year		
9	Habit of Wearing		
	protective Glasses	25 (21.00)	NA
	Females	27 (22.68)	NA
	Males		
10	Computer Vision		
	Syndrome	40 (33.61)	NA
	Females	42 (35.29)	NA
	Males		

27 (22.68 %) male subjects and 25 (21%) female subjects were in the habit of wearing protective glasses. In the study cohort, the mean duration of mobile phone usage was higher in male subjects 52(43.69%) than female subjects 40 (33.61%).A higher degree of mean values were observed in exposed subjects than controls. The mean value of micronuclei in exposed male and female subjects was 5.63±1.20 and 5.31±0.13 respectively as against the control male and female subjects with mean values as 3.87±1.20 and 3.28±0.01 respectively. The mean value of binucleated cells in exposed male and female was 3.88±0.25 and 3.48±0.41 respectively. On the other hand, the control male and female subjects revealed a lower frequency of binucleated cells as indicated by a mean value of 2.45±0.07 and 2.27±1.01.

Smoking and duration of exposure as hours per day and in number of years had a significant effect on buccal cells in the exposed subjects in the present study. The results of this study indicated that the cytogenetic damage of buccal epithelial cells in the mobile phone and computer users increased significantly, as compared with controls. The exposed male subjects indicated a higher degree of karryorhexis cell 7.42±0.25 and karryolysis cell 13.71±0.50 when compared to control subjects both male (6.84±1.21) and female (9.56±0.11) respectively. Male subjects had a higher degree of nuclear abnormalities than the females. It was observed that subjects with exposure period of more than six years had a higher degree of nuclear abnormalities than subjects with less than 5 years of exposure. The results obtained from this study on exfoliated

Table 2. Cytological observation for exposed (*n*=119) and controls (*n*=101

Group	Subject	Cytological Observation				
		W D	MN	BN	KR	KL
Controls subjects						
Male						
Female	49	NA	3.87±1.20	2.45±0.07	6.84±1.21	9.56±0.11
	52	NA	3.28±0.01	2.27±1.01	6.01±0.24	9.14±1.32
Exposed subjects						
Male						
Female	63	8.25 ± 1.27	5.63±1.20*	3.88±0.25*	7.42±0.25*	13.71±0.50*
	56	6.13 ± 2.54	5.31±0.13*	3.48±0.41*	7.02±1.20*	13.02±0.14*
Control subjects						
Group 1						
Group2	51	NA	4.48±0.08	3.25±0.16	6.84±0.58	11.45±0.24
Group3	18	NA	4.26±0.17	2.91±0.01	5.58±0.07	11.21±0.34
Group4	86	NA	3.86±0.84	2.84±0.67	5.01±0.21	6.85±0.37
	47	NA	3.82±0.10	2.35±0.68	4.98±1.05	6.01±0.11
Exposed subjects						
Group 1						
Group2	62	6.52±0.08	4.75±1.32*	3.62±1.02*	7.11±1.27*	12.01±0.07*
Group3	17	6.45±1.21	4.50±1.01*	3.11±0.87*	6.81±0.27*	11.47±0.14*
Group4	97	5.01±017	4.11±1.24*	3.09±1.10*	5.27±0.08*	7.47±0.17*
	62	5.51±1.15	3.91±0.35	2.55±0.59	5.15±1.24	6.14±0.85

WD: Working Duration, NA: Not Applicable, MNC: Micronuclei; BN: Binucleated cells; KR: Karyorrhectic cells, KR: Karyolytic cells.

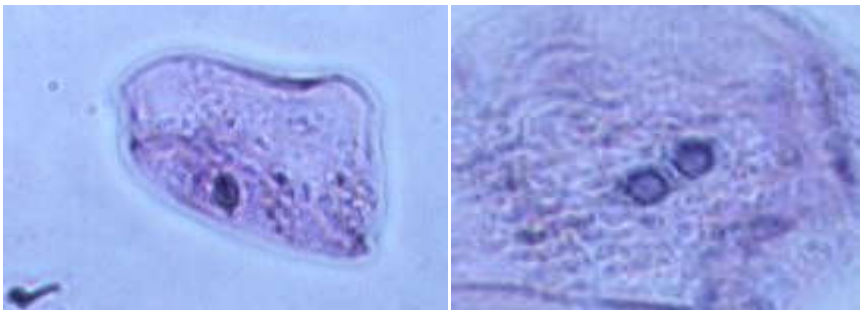
Exposed subjects
Group 1: smokers +cell phone users+ computer users
Group 2: smokers + non cell phone users+ computer users
Group 3: non smokers +cell phone users+ computer users
Group 4: non smoker +non cell phone users +computer users
Control subjects
Group 1: smokers +cell phone users+ non computer users
Group 2: smokers + non cell phone users+ non computer users
Group 3: non smokers +cell phone users +non computer users
Group 4: non smoker +non cell phone users +non computer users

* Significant at 0.05 level (Student's t-test).

buccal cells indicate that exposed male individuals have a significant increase in number of MN, KR and KL. Exposed female and control female have variations in the values (Table 2). The results of the cytological observations on MN, BN, KL and KR of the exposed group revealed higher frequency than the control group (Table 2).

fatigue and difficulty in refocusing the eyes. Thus, an inexpensive, non invasive method to assay the degree of exposure with its causative effect becomes imperative. To our knowledge, this is the first study that has adopted nuclear buccal cytome assay to estimate the degree of radiation exposure among computer and mobile phone users.

Figure 1. Detailed description of the various cell types observed in the present study.



A) Micronuclei B) Binucleated cell

C) Karyorrhetic cell D) Karyolytic cell.

Micronuclei

Micronuclei are identified with presence of main nucleus and one or more smaller nuclei (micronuclei) in the cells. The micronuclei are usually round or oval in shape and their diameter may range from 1/3 to 1/16, the diameter of the main nucleus.

Binucleated cells

Binucleated cells have two nuclei that are adherent to each other. This is indicative of failed cytokinesis.

Karyorrhectic cells

Karyorrhetic cells have dense network of nucleochromatin elements that lead to fragmentation and disintegration of the nucleus.

Karyolytic cells

In karyolytic cells, the nucleus is devoid of DNA and appears to have no nuclei. This indicates very late stage in cell death process. It has a cloudy appearance with no distinct features.

DISCUSSION

The computer vision syndrome comprises common complications like reddening and drying of eyes, headaches, blurred vision, neck pain, eye strain and

However, most of them, if not all are mobile phone users and some of the male executives in this study were habitual smokers. These workers did not adopt any protective measures like CRT screen shields and protective eye glasses. Most of the male staff worked on night shifts, and hence a

slightly higher degree of nuclear anomalies were observed in men than women. The International Agency for Research on Cancer IARC (2002) has classified low frequency EM field as a possible carcinogen - a categorization that necessarily implies that low EM field may promote DNA damage and hence may be genotoxic. Andersson, 1996 and Kishner *et al.,* 1998 reported that female workers exposed to VDU presented obstetric complications, besides skin, ocular and CNS diseases. Gangi *et al.,* 2000 detected an increased incidence of skin and central nervous system (CNS) alterations among microcomputer workers. In our study, we also detected computer vision syndrome in 68 % (n=82) of the exposed group which may be as a result of continuous exposure to EMF emitted by computer monitor. Estecio *et al.,*, 2002 reported that microcomputer's workers who are exposed to radiation had two times more chromosomal aberrations in cultured lymphocytes than control individuals. Carbonari *et al.,* 2005 indicated significant cytogenetic damage by micronuclei assay in computer workers. Lakshmi *et al.,* 2009 reported that computer users exposed to radiation for more than 10 years showed higher induction of DNA damage and increased frequency of micronuclei and micronucleated cells.

Other factors such as poor dental health were ruled out in our study, as the recruited subjects had no history of dental ailments, dental X rays or amalgam filling in the last 2 years. Bloching *et al.,* 2008 report shows a higher degree of MN in buccal smears of subjects with periodontal diseases. Schweikel *et al.,* 2005 reported that nuclear anomalies in *in-vitro* studies in extracts of fine common dental composites. Our study recruited only subjects without dental fillings / radiation therapies since it is considered that DNA damage, higher degree of nuclear anomalies and binucleate is possible due to such therapies. Rashmi *et al.,* 1984 reported that buccal cytome assay can be adopted for risk assessment of oral cancer in patients with precancerous states of the oral cavity. In spite of the extensive increase in the number of computer users within the last few years, very little is known and there are very few reports about the effects of prolonged exposure that is experienced by computer and mobile phone users. Hence this study was carried out to investigate the effect of occupational electromotive force (EMF) exposure on DNA damage and frequency of micronuclei on buccal cytome assay of the computer users. Jaschinski *et al.,* 1999 and Jaschinski 2002 reported that the distance between the operators and the computer screen is another important factor. Recent empirical studies suggest that viewing distances of 35–40 inches may actually reduce the incidence of visual strain. The radiation screen also improves display clarity and reduces static charges, and includes protection against magnetic fields, which some medical researchers believe to be an important factor in biological changes. Computer users mostly used mobile phones for communications. In this study the usage of mobile phones is higher in males than female subjects this may be one of the reasons for increased frequency of cells with micronuclei. Yadav *et al.,* 2002 indicated that mobile phone radiations cause *in vivo* effects on the frequency of micronucleated cells in the mobile phone users. Gandhi *et al.,* 2002 reported that a higher frequency of micronucleated buccal cells and cytological abnormalties in cultured lymphocytes of mobile phone users indicates the genotoxic responses due to mobile phone use.

The present study also indicated an increased frequency of micronuclei in both male and females. A review on cell phones and tumor risk by Hardell *et al.,* 2007 and Gadhia *et al.,* 2003 revealed increased risk for brain tumors reported that a significant increase in Chromosomal aberrations (CA) and Sister Chromatid Exchange (SCE) among mobile phones users than that in control group. In this study also the cytogenetic damage as revealed by buccal cytome assay indicates a higher frequency of clastogenecity in subjects with exposure for more than six years. Similar studies reported that more than 10 years of mobile phone exposure is associated with high risk of brain tumor and increased risk for acoustic neuroma and glioma (Lahkola *et al.,* 2007 and Hardell *et al.,* 2007). The risk is highest for ipsilateral exposure. To conclude, our preliminary results strongly suggest that computer and mobile phone users, exposed to EM radiation presented a slightly increased frequency of cells with micronuclei, which they are exposed at work and however, extensive studies are needed to evaluate biological

damage at different levels and duration of exposure to computers and mobile phones. Genotoxic evaluation becomes imperative and is a necessary measure to ensure environmental quality and occupational health of individuals.

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REFERENCES

Abhay Singh Yadav, Manoj Kumar Sharma. 2008. Increased frequency of micronucleated exfoliated cells among humans exposed *in vivo* to mobile telephone radiations. Mut.Res., 650: 175 – 180 .

Andersson B. 1996. A cognitive-behavioral treatment of patients suffering from "electric hypersensitivity". Subjective effects and reactions in a double-blind provocation study. J Occup. Environ Med., 38:752-758.

Blatter BM, Bongers PM. 2002. Duration of computer use and mouse use in relation to musculoskeletal disorders of neck or upper limb. Int J of Ind Ergonomics.30: 295–306.

Blehm C ,Vishnu S, Khattak A., Mitra., S and Yee RW. 2005. Computer Vision syndrome: A review. Survey of Opthalmology 50(3):253-262.

Carbonari K, Goncalves L, Rpth D, Moreira P, Fernandez R, Roth MGM. 2005. Increased micronucleated cell frequency related to exposure to radiation emitted by computer cathode ray tube video display monitors, Genet. Mol. Biol., 28: 469–474.

Catherine Cook, Robin Burgess-Limerick, Sungwon Chang. 2000. The prevalence of neck and upper extremity musculoskeletal symptoms in computer mouse users Int J of Ind Ergonomics 26: 347 -356.

Estecio MRH and Silva AE. 2002. Chromosome abnormalities caused by computer video display monitor's radiation. Rev. Saude Publica.,36: 330 – 336.

Flodgren G, Heidena M, Lyskova E, Crenshawa A G. 2007. Characterization of a laboratory model of computer mouse use—Applications for studying risk factors for musculoskeletal disorders. Appl. Ergonomics., 38: 213–218.

Gadhia PK, Shah T, Mistry A, Pithawala M and Tamakuwala D. 2003. A Preliminary study to assess possible chromosomal damage among users of digital mobile phones. Electromag. Biol. Med., 22: 149-159.

Gangi S and Johansson O. 2000. A theoretical model based upon mast cells and histamine to explain the recently proclaimed sensitivity to electric and / or magnetic fields in humans. Med. Hypoth., 54: 663 – 671.

Grace Pui Yuk Szeto, Leon Melville Straker, Peter Bruce O'Sullivan. 2009. Neck shoulder muscle activity in general and task-specific resting postures of symptomatic computer users with chronic neck pain. Manual Therapy.,14: 338 – 345.

Gursatej Gandhi and Prabhjot Singh. 2005Cytogenetic damage in mobile phone users: Preliminary Data. Int J Hum. Genet., 5(4): 259-265.

Helmut Schweikel, Karl-Anton Hiller, Carola Bolay, Marion Kreissl, Wetscheslaw Kreismann, Agathe Nusser, Stefanie Steinhauser, Janusz Wieczorek,Rudolf Vasold, Gottfried Schmalz. 2005. Cytotoxic and mutagenic effects of dental composite materials, Biomaterials., 26: 1713–1719.

IARC. 2002. Extremely Low-Frequency Electric and Magnetic Fields: IARC Monographs on the Evaluation of Carcinogenic Risk to Humans, International Agency for Research on Cancer, Lyon vol 20.

Ivancsits S, Diem E, Pilger A, Ruiger HW and Jahn O. 2002. Induction of DNA strand breaks by intermittent exposure to extremely low frequency electromagnetic fields in human diploid fibroblasts. Mut.Res.,519: 1 – 13.

Jaschinski W, Heuer H and Kylian H. 1999. A procedure to determine the individually comfortable position of visual displays relative to the eyes. Ergonomics., 42(4): 535 – 549.

Jaschinski W. 2002. The proximity-fixation-disparity curve and the preferred viewing distance at a visual display as an indicator of near vision fatigue. Optometry and Vision Science.,79(3): 158–169.

Kisner RS and Federman DG. 1998. Video display terminals: Risk of electromagnetic radiation. Southern Med J., 91:12-16.

Lahkola A, Auvnen A, Raitanen J, Schoemaker MJ, Christensen HC, Feychting M, Johansen C, Klaeboe L, Lonn S, Swerdlow AJ, Tynes T, Salminen T. 2007. Mobile phone use and risk of glioma in 5 North European countries, Int. J. Cancer., 120 (8): 1769–1775.

Lakshmi NK, Tiwari R, Bhargava SC and Ahuja YR. 2009. Investigations on DNA damage and frequency of micronuclei in occupational exposure to electromagnetic fields (EMFs) emitted from video display terminals (VDTs).Genet. Mol. Biol., 33(1): 154-158.

Lennart Hardell , Carlberg M, Söderqvist F, Mild KH and Morgan LL. 2007. Long-term use of cellular phones and brain tumors - increased risk associated with use for > 10 years. Occup. Environ. Med., 64: 626-632.

Lennart Hardell, Kjell Hansson Mild, Michael Carlberg and Fredrik Söderqvist. 2007. Tumour risk associated

with use of cellular telephones or cordless desktop telephones Occup. Environ. Med., 64: 626–632.

Li CY and Theriault RS. 1997. Residential exposure to 60 Hz magnetic fields and adult cancers in Taiwan. Epidemiol., 8: 25-30.

Marc Bloching, Waldemar Reich, Johannes Schubert, Tamara Grummt and Annett Sandner 2008. Micronucleus rate of buccal mucosal epithelial cells in relation to oral hygiene and dental factors. Oral Oncology, 44: 220 – 226.

Majer BJ, Laky B, Knasmuller S and Kassie F. 2001. Use of the micronucleus assay with exfoliated epithelial cells as a biomarker for monitoring individuals at elevated risk of genetic damage and in chemoprevention trials. Mut. Res., 489: 147 – 172.

Masayuki Tatemichi, Tadashi Nakano, Katsutoshi Tanaka, Takeshi Hayashi, Takeshi Nawa, Toshiaki Miyamoto, Hisanori Hiro, Minoru Sugita. 2004. Possible association between heavy computer users and glaucomatous visual field abnormalities: a cross sectional study in Japanese workers. J Epidemiol Community Health, 58:1021–1027.

Nakaishi H and Yamada Y. 1999. Abnormal tear dynamics and symptoms of eyestrain in operators of visual display terminals. Occup. Environ. Med., 56:6-9.

Rashmi saran, Ravindra Tiwari K, Penagaluru Paradhnandan Reddy and Raj Ahuja. 1984. Risk assessment of oral cancer in patients with precancerous states of the oral cavity using micronucleus test and challenge assay. Oral Oncology, 44: 354 – 360.

Sen A and Richardson S. 2002. Some controversies relating to the causes and preventive management of "Computer Vision Syndrome.

Stella C Chiemeke Member, IAENG, Allen E. Akhahowa and Olajire B. Ajayi. 2007. Evaluation of Vision-Related Problems amongst Computer Users: A Case Study of University of Benin, Nigeria Proceedings of the World Congress on Engineering Vol I.

Stitch HG and Rosin MP. 1984. Micronuclei in exfoliated human cells as tool for studies in cancer risk and cancer intervention. Cancer Lett., 22:241 - 253.

Thorn S, Søgaard K, Kallenberg LAC, Sandsjo L, Sjøgaard G, Hermens HJ, Kadefors R, Forsman M. 2007. Trapezius muscle rest time during standardised computer work – A comparison of female computer users with and without self-reported neck/shoulder complaints. Journal of Electromyography and Kinesiology., 17: 420–427.

Titenko-Holland N, Moore LE and Smith M T. 1994. Measurement and characterization of micronuclei in exfoliated human cells by fluorescence in situ hybridization with a centromeric probe. Mut. Res., 312: 39 – 50.

Tolbert PE, Shy CM, Allen JW. 1992. Micronuclei and other nuclear abnormalities in buccal smears: methods and development. Mut. Res., 271: 69 -77.

Verschaeve L. 2005. Genetic effects of radiofrequency radiation (RFR) .Toxicol. Appl. Pharmacol., 207:S336-S341.

Wertheimer N and Leeper E. 1979. Electrical wiring configurations and childhood cancer. Am J Epidemiol., 109: 273 – 284.

Zheng Yan, Liang Hu, Hao Chen, Fan Lu. 2008. Computer Vision Syndrome: A widely spreading but largely unknown epidemic among computer users. Computers in Human Behavior, 24: 2026–2042.

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